FISHERY RESEARCH

WHIRLING DISEASE RESEARCH REPORT: AN EXPOSURE TRIAL TO DETERMINE THE PROXIMITY OF *MYXOBOLUS CEREBRALIS* INFECTIVITY TO THE PRODUCTION FACILITIES AT MACKAY STATE FISH HATCHERY

FINAL REPORT
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ABSTRACT

The presence of fish in the Mackay Hatchery settling pond that are infected by *Myxobolus cerebralis*, the causative agent of salmonid whirling disease, has been known since 1988. However, it was not known whether such fish became infected while in the pond, or if they were infected elsewhere before migrating into the pond. Live-boxes containing susceptible juvenile rainbow trout *Oncorhynchus mykiss* were placed in locations on the hatchery, in the settling pond, and in Warm Springs Creek below the pond to determine sites where fish would become infected, thus indicating where the parasite has become established. Groups of fish were exposed for eleven days, and then held at the Eagle Fish Health Laboratory for 100 days to allow for *M. cerebralis* spore development. No infected fish were detected from groups exposed in the large raceway headbox or tailrace, or from a group of fish held as controls in a vat inside the hatchery building. Infected fish were detected in groups of fish exposed at two different locations in the settling pond and in the group from Warm Springs Creek. The very close proximity of the parasite to the production facilities of the hatchery should prompt actions to preclude contamination of production fish.

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INTRODUCTION

Myxobolus cerebralis (MC), the causative agent of salmonid whirling disease, was first detected in trout from Warm Springs Creek below Mackay Hatchery in 1988 (Eagle Fish Health Laboratory database). Those original samples included fish from the hatchery's settling pond. Another fish from the settling pond tested positive for the parasite in 1995. Production fish from the hatchery raceways have routinely been tested and the parasite has never been detected. It has always been assumed that the design of the hatchery, (i.e., covered springs and concrete raceways), would not allow completion of the MC life cycle, thus preventing infection of production fish. Another assumption has been that the positive fish in the settling pond were either infected in the dirt-bottom pond after escaping from the production raceways, or had migrated up into the pond after being infected in lower Warm Springs Creek or in the Big Lost River (both of which have been demonstrated to be infective). However, no effort has been made to test either of these assumptions. It would be to our advantage to gain knowledge of the physical proximity of infectivity to the hatchery. Such knowledge could be used to assess the probability of MC contamination in the production portions of the hatchery and the risk that fish may become infected at levels below our ability of detection. Decisions could then be made regarding the need to change hatchery designs or management practices to prevent such contamination.

OBJECTIVES

There were two purposes to this trial. First, to determine if MC infectivity can be detected in the Mackay Hatchery water source or production raceways. Second, to detect where, in relationship to the Mackay Hatchery production raceways, infection by MC naturally occurs.

STUDY SITE

Mackay Hatchery is located in Custer County Idaho, approximately 18.5 km northwest of the town of Mackay (UTM 12T, 273067E, 4873261N). Locations selected for the live-boxes were (Figure 1):

1. The large raceway headbox, as close as practical to the spring source.
2. The large raceway tailrace, above the barrier screen.
3. The settling pond immediately below the culvert from the small raceways.
4. The settling pond, along the northeast concrete wall.
5. Warm Springs Creek, approximately 120 meters below the settling pond.

In addition, a sixth group was held in the hatchery vat building to serve as a control for the outside exposure groups.

METHODS

Cylindrical, aluminum live-boxes were disinfected with chlorine and brought to Mackay. Stowaway XTI temperature loggers were placed in three boxes (locations 1, 4, and 5, above) to record water temperatures at 30-minute intervals during the exposure period.

Eyed triploid rainbow trout *Oncorhynchus mykiss* eggs were acquired from Hayspur Hatchery, hatched, and reared inside the hatchery building at Mackay Hatchery. When the fish averaged 0.83 grams each, sentinel groups of 50 were randomly selected from the main lot and placed in 5 live-boxes on May 20, 2002. Another group was kept in a vat inside the hatchery building as a control for the sentinel groups.

The sentinel groups remained in the live-boxes for eleven days, then were retrieved and transported to the Eagle Fish Health Laboratory. Groups were reared in separate tanks in the wet laboratory for 108 days using 13°C well water. All mortalities were removed and frozen. All surviving fish were sacrificed Sept. 9, with individual heads removed, split, and pooled in groups of 5. One set of pooled half-heads was tested using the pepsin/trypsin digest (PTD) method, and the other set was frozen as backup. Nested polymerase chain reaction (PCR) testing was done on selected sample pools from two groups that tested positive by PTD. This test amplifies and identifies species-specific segments of genetic material, and was done to confirm that the spores observed in PTD were indeed MC and not another species of *Myxobolus*.

RESULTS

The PTD tests (Table 1) detected no spores from fish in the control group, or from sentinel fish from Sites 1 and 2 (large headrace and tailrace). Spores were detected by PTD in groups from both sites in the settling pond (in seven of seven 5-fish pools from Site 3, and in two of eight 5-fish pools and zero of one 2-fish pool from Site 4). Five individuals from Site 3 were tested by PCR and MC genetic material was detected from all five. Spores were also found in all pools (eight 5-fish and one 2-fish) from Site 5, with PCR confirmation of MC from five of five individuals.

Water temperatures were recorded at Sites 1, 4, and 5. Mean water temperatures for the exposure period were 11.43°C at Site 1 (large headbox), 11.39°C at Site 4 (large tailrace), and 11.26°C at Site 5 (Warm Springs Creek).

DISCUSSION

The objectives of the trial were met in that we detected MC infections from naïve fish exposed in the Mackay Hatchery settling pond and in the creek directly downstream, but not from fish exposed in the headrace or tailrace of the large raceways or in the hatchery vat building. These findings may be interpreted to mean that the parasite does complete its full life cycle within the confines of the settling pond, but not within the production portions of the hatchery.

There was an obvious difference in detected infections between the two sites within the settling pond (Table 1). A reasonable explanation might be the difference in water velocity between the two sites. Site 2 was in a quiescent zone between the outflows for the large and smaller raceways, while Site 3 was directly in the main current from the large raceways. Actual velocity was not measured, but a conservative estimate would be at least 1.5-2 meters/sec at Site 3. Triactinomyxons (TAM) drift passively in the water, and increased current may have an effect upon a TAM's ability to physically attach to a host fish.

The results of this trial suggest that MC has not become established within the production areas of the hatchery, and it is reasonable to assume that it will not become so as long as hatchery operations are not radically changed. The springs are covered and do not provide habitat for feral fish or for *Tubifex tubifex* worms, the alternate host for MC. The hatchery building and both sets of smaller raceways are supplied with water through buried pipelines, while the large raceway headrace is covered. All raceways are routinely cleaned, and dead fish are removed. A screen has been placed in the large raceway tailrace to exclude settling pond fish, while the effluent from both banks of small raceways flow through buried pipelines that provide no habitat for either fish or *T. tubifex*. Thus, there are several factors that prevent completion of the life cycle of MC and establishment of the parasite within the actual production facilities.

There are other potential sources of contamination that could lead to infected fish within the production lots. The movement of spores into the production raceways is of little concern, since spores do not directly infect fish, and the hatchery environment does not provide habitat for *T. tubifex*. But the infective TAM stage of the parasite could be introduced into production water by animal vectors. For example, mammals such as mink, otters, muskrats, or humans might move directly from the settling pond to a raceway. Water on fur, hands, or equipment might contain TAMs that could wash into the raceway. Fish-eating birds, such as herons or seagulls could similarly carry water and TAMs. The prevalence and intensity of any resulting infections would almost certainly be far below the detection level of standard testing, and no signs of clinical disease would be likely from such light infections. The probability of such contamination is low, but not unprecedented in IDFG records. Specifically, an MC-infected fish was detected from a group of age 1+ rainbow trout at Hayspur Hatchery in 1999 (Eagle Fish Health Laboratory database). Hayspur Hatchery's circumstances are very similar to Mackay Hatchery, in that fish are reared in concrete raceways with an enclosed well water source, and MC is known to be present in earthen ponds on both sides of the facility. The infected fish was in a raceway that was frequently visited by a family of otters coming directly out of one of those ponds (Bob Esselman, Hatchery Manager, personal communication).

RECOMMENDATIONS

Results of this study and routine sampling of production lots do not indicate the presence of MC in the production lots of fish at Mackay Hatchery. Yet, this study does demonstrate the extremely close proximity of the parasite to the production facilities. There is a potential risk of MC contamination of production fish at levels below our ability to detect, particularly to those fish held outside for the longest period of time. This risk should be taken into consideration whenever new programs for Mackay Hatchery are contemplated or new sites for stocking Mackay Hatchery fish are suggested. Distribution of fish reared solely in the hatchery building, where contamination is least likely, would entail the least risk.

I strongly recommend that the hatchery manager and assistant manager continue to make a commitment to training for all hatchery personnel, particularly seasonal temporaries, in the steps necessary to prevent introduction of the parasite to the production facilities. The disastrous impact of carelessness on Idaho Fish and Game Department programs cannot be over-emphasized.

I also recommend that the construction of predator exclusion structures around the “Small” and “Hole” sets of raceways be funded and implemented. Overhead netting to exclude birds would be the easiest to build and maintain, and would have the added advantage of preventing depredation losses. The effectiveness of such structures could be evaluated before constructing larger, more expensive structures over the large raceways. Mackay Hatchery produces excellent fish and further efforts to prevent MC contamination would greatly increase the confidence level in that quality product.

ACKNOWLEDGMENTS

I wish to acknowledge the Mackay Hatchery crew, specifically the Hatchery Manager Phil Coonts, and Assistant Manager Mick Hoover, for their assistance in starting the fish and in placing the live-boxes. Fish Health Technologists Sharon Landin, Carla Hogge, and Roberta Scott processed and analyzed the samples at the Eagle Fish Health Laboratory. Elaine Cavenaugh was very helpful in the preparation of this manuscript.

Table 1. Pepsin/trypsin digest test results for *Myxobolus cerebralis* spores from rainbow trout fry *Oncorhynchus mykiss* exposed to waters in and around Mackay Hatchery, Custer County, Idaho. May 20 to May 31, 2002.

Site	Site Location	Total No. of fish tested	No. of Pools	No. of Positive Pools	PCR Confirmed
Control	Hatchery building	50	10x5	0	NA
1	Large raceway headrace	47	9x5 + 1x2	0	NA
2	Large raceway tailrace	41	7x5 + 1x6	0	NA
3	Settling pond; in quiescent zone near small outflow	35	7x5	7	Yes 5 of 5
4	Settling pond, against concrete wall	42	8x5 + 1x2	2(x5)	NA
5	Warm Springs Creek	45	9x5	9	Yes 5 of 5

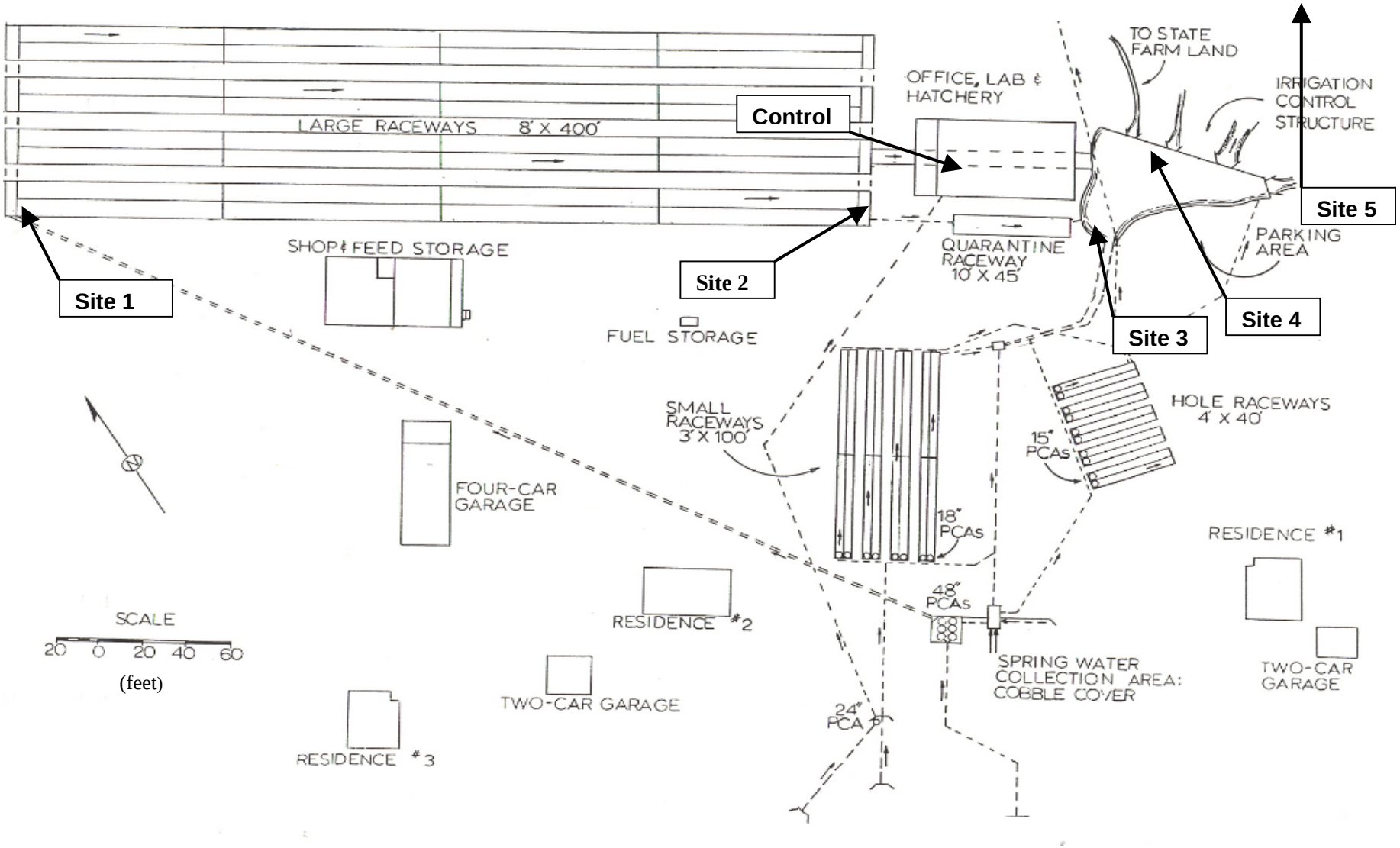

Figure 1. A schematic diagram of Idaho Department of Fish and Game's Mackay Hatchery showing live-box site locations for a whirling disease exposure trial done May 20 to May 31, 2002. Drawn by Mick Hoover, Assistant Hatchery Manager.

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